AD_____

Award Number: W81XWH-11-1-0111

TITLE: Nano-Engineered Drug Combinations for Breast Cancer Treatment

PRINCIPAL INVESTIGATOR: Joerg Lahann, Ph.D.

CONTRACTING ORGANIZATION: University of Michigan
Ann Arbor, MI, 48109

REPORT DATE: June 2012

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	**2. REPORT TYPE**	**3. DATES COVERED**
June 2012	Annual	15 May 2011 – 14 May 2012

4. TITLE AND SUBTITLE

Nano-Engineered Drug Combinations for Breast Cancer Treatment

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-11-1-0111

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Joerg Lahann, Ph.D.

E-Mail: lahann@umich.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

University of Michigan
Ann Arbor, MI, 48109

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

We hypothesis that nanoparticles can increase the efficacy of combination therapies for cancer. The project aims to explore nanoparticle-based transport vehicles for effective delivery of two different cancer drugs. In particular, we aim to use two breast cancer drugs, which enhance each other's efficacy and use them as a model drug pair for the development of novel dual-drug nano-therapeutics. The research to date has been focused on extending existing electrohydrodynamic co-jetting technology in the Lahann lab towards the simultaneous release of two independent small molecule drug surrogates.

15. SUBJECT TERMS
None provided.

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			USAMRMC
U	U	U	UU	10	**19b. TELEPHONE NUMBER** *(include area code)*

Nano-engineered Drug Combinations for Breast Cancer Treatment

Progress Report

Table of Contents

Introduction

Multicompartmental nanocarriers containing multiple cancer therapeutics can be fabricated through the electrohydrodynamic (EHD) co-jetting procedure.[1] The EHD process has been developed in the Lahann laboratory and has been established as a reliable, reproducible, and versatile method for the fabrication of monodispersed anisotropic particles and fibers.[2-6]

In the EHD process,[7] multiple polymer solutions are flown in a laminar regime through syringes tipped with metal needles. The needles are connected to a high voltage source, which is grounded via a metal collector placed beneath the syringes. As a DC voltage is applied to the needles, the solutions at their tip form into a Taylor cone.[8] At the end of this Taylor cone, a thin, high-speed jet is formed that travels toward the grounded electrode. The jet exiting the tip of the Taylor cone becomes thinner and eventually breaks into small droplets. During this process, the solvents evaporate rapidly, leaving behind solid anisotropic particles that are collected on a counter electrode.[9] Due to the rapid evaporation of the solvents and the laminar flow regime used, the polymers do not have sufficient time to mix, and, thus, result in particles with distinct compartments. In these particles, the number of compartments is determined based on the number of individual needles originally used.[5, 9]

Progress Report of Research Tasks.

Low molecular weight molecules, such as dyes and therapeutics, can be incorporated into multicompartmental particles by adding them to the polymer solutions used for jetting. However, unlike the polymers that are high molecular weight (on the orders of tens of kDa) and do not have sufficient time to mix during the jetting, the low molecular weight molecules (less than a kDa) can diffuse and mix with the other jetting solutions much faster. While this is not an issue when the molecules are used at low concentrations (less than 5% w/w of the polymer content), it can become a major problem when a high loading of a therapeutic is used. In order to combat this problem, triphasic

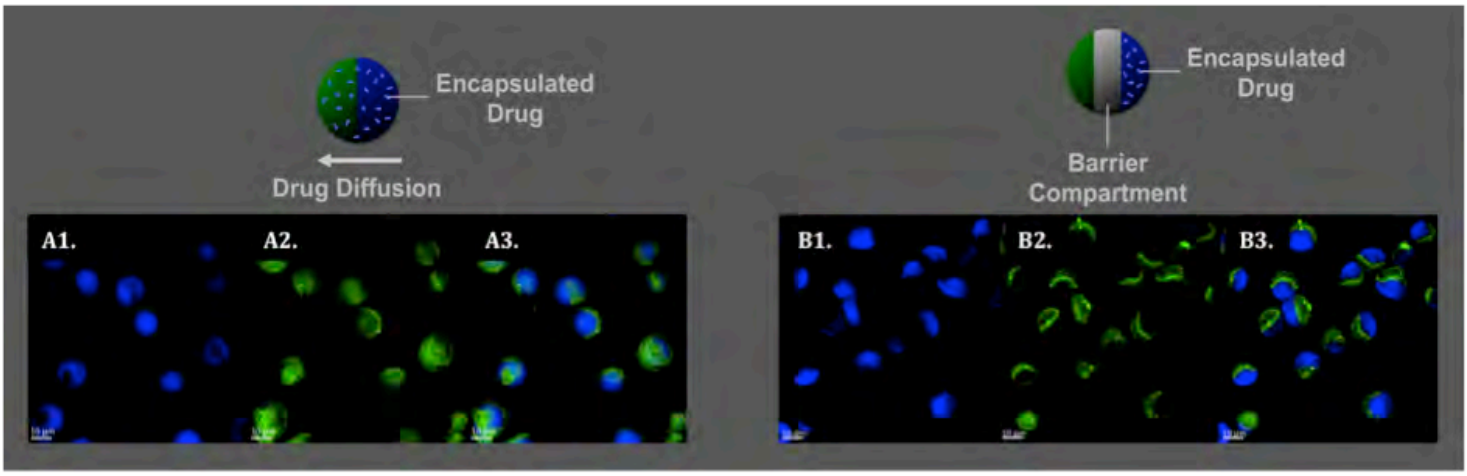


Figure 1: High loading of therapeutics can be compartmentalized in particles by using a barrier compartment. A. In particles without a barrier compartment, the drug (blue) diffuses to the second compartment (containing a green dye). B. In particles with a barrier compartment, the drug is encapsulated only in one compartment and does not travel to the second compartment (green).

particles were fabricated with a middle compartment that could act as a 'barrier'. This 'barrier compartment' is composed of relatively hydrophobic, high molecular weight polymers that act as a deterrent to the diffusion of molecules to the other compartments. While the low molecular weight dyes/therapeutics have sufficient time to mix into the other compartment in bicompartmental systems (**Figure 1.A**), they do not have enough time to diffuse through triphasic particles with this 'barrier compartment', and thus stay compartmentalized (**Figure 1. B**). We were able to show compartmentalization of a cancer therapeutic, Irinotecan, up to a high loading of 25% w/w of the polymer in one compartment.

Nanoparticles containing two different low molecular weight entities in separate compartments were fabricated using the barrier compartment method and tested via release studies. The particles were 800 nm on average and contained a cancer therapeutic, Irinotecan, in one compartment, and a low molecular weight dye, Rhodamine, in the other. Release studies with the particles showed distinct release of the two molecules (**Figure 2**). Here the difference in release profiles were achieved based on the molecular weight of the polymers used (44 kDa for Irinotecan and 50-75 kDa for Rhodamine) and the lactide-to-glycolide ratio of the polymers (50:50 for Irinotecan and 85:15 for Rhodamine), both of which determine the rate of the degradation of the polymer. As a result, the compartment with the lower molecular weight and higher ratio of glycolide released its therapeutic load (Irinotecan) first.

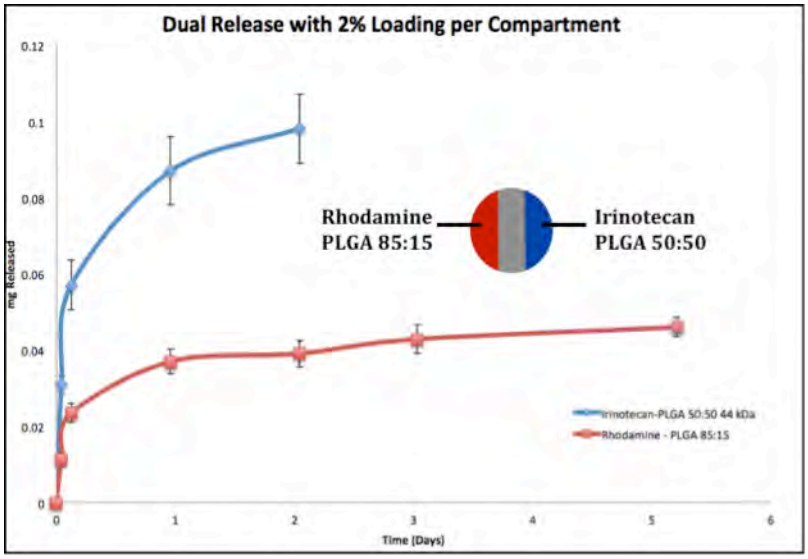


Figure 2: Dual distinct release from multi-compartmental particles with a barrier compartment. A low molecular weight dye (Rhodamine-Red) is encapsulated in one compartment and a low molecular weight drug (Irinotecan-Blue) in the other.

The use of different polymers, or different ratios of the same polymers, can be explored to accurately tune-in release kinetics. A hydroxyl-modified polylactide (PLA-OH) has been synthesized in the Lahann laboratory with a fast degradation period of several hours. Drug loaded particles with PLGA in one compartment and different percentages of PLA-OH (0, 10, 50, or 100%) in the other compartment were fabricated. Depending on the amount of PLA-OH used, the release profile of the encapsulated therapeutic, Irinotecan, could be tuned-in: as the level of PLA-OH increased, so did the release rate of Irinotecan (**Figure 3**).

In order to take advantage of this tunability, particles with two low molecular weight molecules, Irinotecan and Rhodamine, were fabricated. Here, one side contained Irinotecan and PLGA, while the other compartment contained Rhodamine and PLA-OH. As expected, the compartment with PLA-OH released its therapeutic load more rapidly than that containing PLGA.

Triphasic particles combining these two methods (barrier compartment and use of rapidly degrading polymers) were fabricated. In this case, two cancer therapeutics, Epirubicin and Irinotecan, were contained in separate sides and released from particles (**Figure 5**). Both Epirubicin and Irinotecan

autofluoresce (red and blue, respectively) and Confocal Laser Scanning Microscopy (CLSM) was used to show that the drugs stay compartmentalized (**Figure 5.A**).

The empty space seen between the two compartments in some of the particles represents the barrier compartment, which did not contain any dyes.

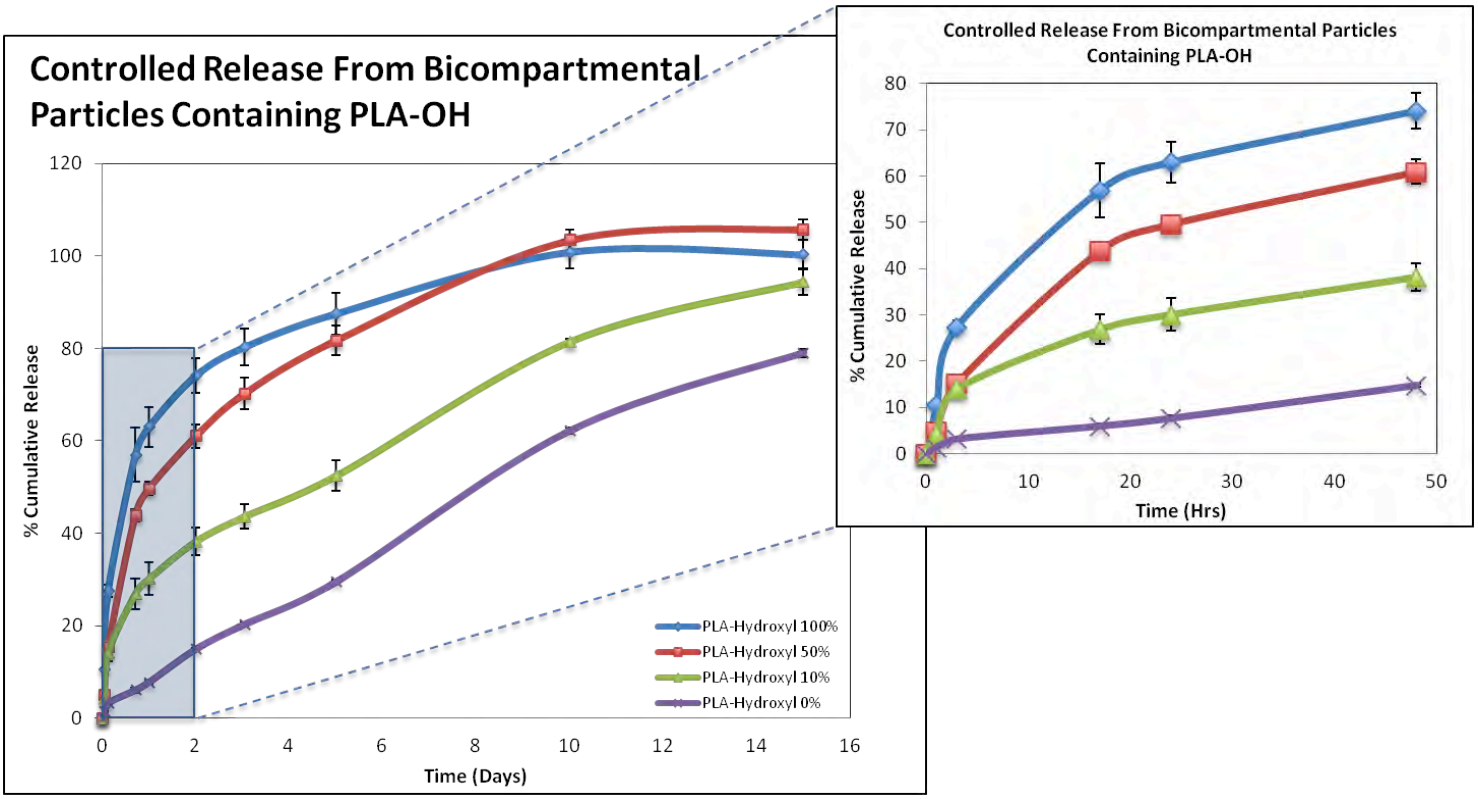


Figure 3: Release of therapeutics can be tuned-in by altering the composition of each compartment. Here, bicompartmental particles with PLGA on one side and different ratios hydroxyl-functionalized PLA (PLA-OH) on the other side were used for release studies. As the ratio of PLA-OH was increased (from zero to 100%), the release of the therapeutic was enhanced.

As shown in **Figure 5.B**, the release of the therapeutics from the particles is distinct and is expected based on previously shown data: Epirubicin contained in the PLA-OH compartment is released faster than Irinotecan that is in the PLGA compartment.

A method to more accurately control the release of therapeutics from particles is the use of materials with on-demand degradation/release characteristics. A number of these polymers have recently been used, and they are typically controlled via an external stimulus (pH, UV, IR, temperature, etc.).[10] Dextran Acetal (DA) is one such

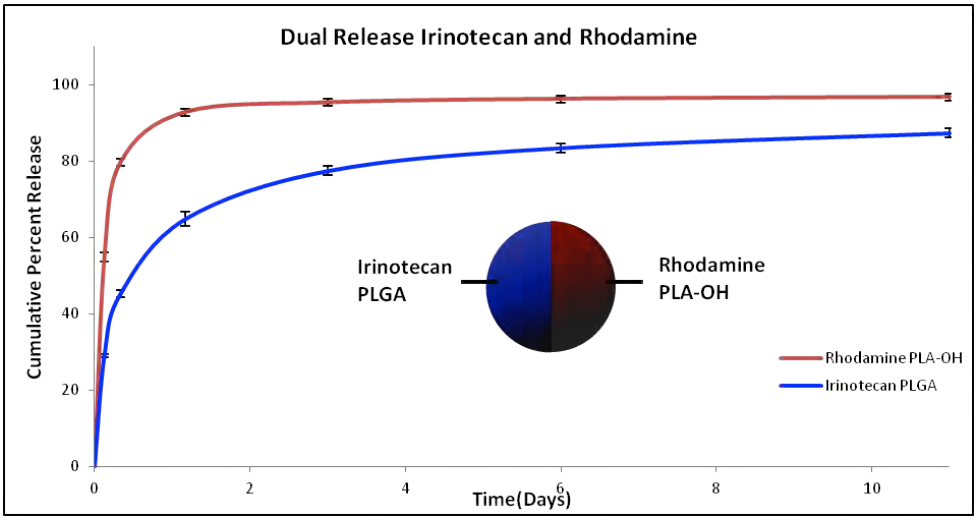


Figure 4: Dual release of therapeutics from bicompartmental particles. Here, one side contains PLGA and Irinotecan, while the other side contains Rhodamine and PLA-OH.

example, as it is pH responsive.[11] At physiological pH, this polymer is stable and water insoluble, but at acidic pH the polymer become deprotected and water-soluble (**Figure 6.A**). Thus, particles made of such a polymer will stay intact in the blood stream, and will only start to dissolve away and release their cargo in environments with an acidic pH (such as the endosome, the extracellular matrix surrounding tumors, and inflamed tissue). The use of such polymer in multicompartmental particles can result in on-demand degradation of one compartment and release of therapeutics.

Particles with 75% w/w DA in one compartment were synthesized and their degradation kinetics were followed via Scanning Electron Microscopy (SEM). It was shown that upon incubation at pH 5, the particles develop visible pores by 5 hrs (**Figure 6.B2**), and start to degrade and completely lose one side by 20 hrs (**Figure 6.B3-5**).

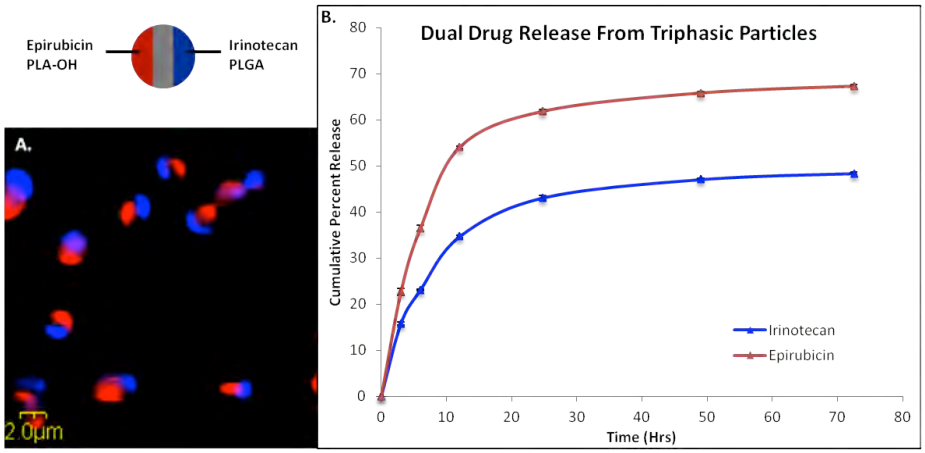


Figure 5: Dual release of cancer therapeutics from bicompartmental particles. Here, one side contains PLGA and Irinotecan and the other contains Epirubicin and PLA-OH. A: Compartmentalized particles containing Epirubicin (autofluorescing red) and Irinotecan (autofluorescing blue) in separate compartments. The barrier compartment does not contain any dyes. B: Dual distinct release of cancer therapeutics from multiphasic particles.

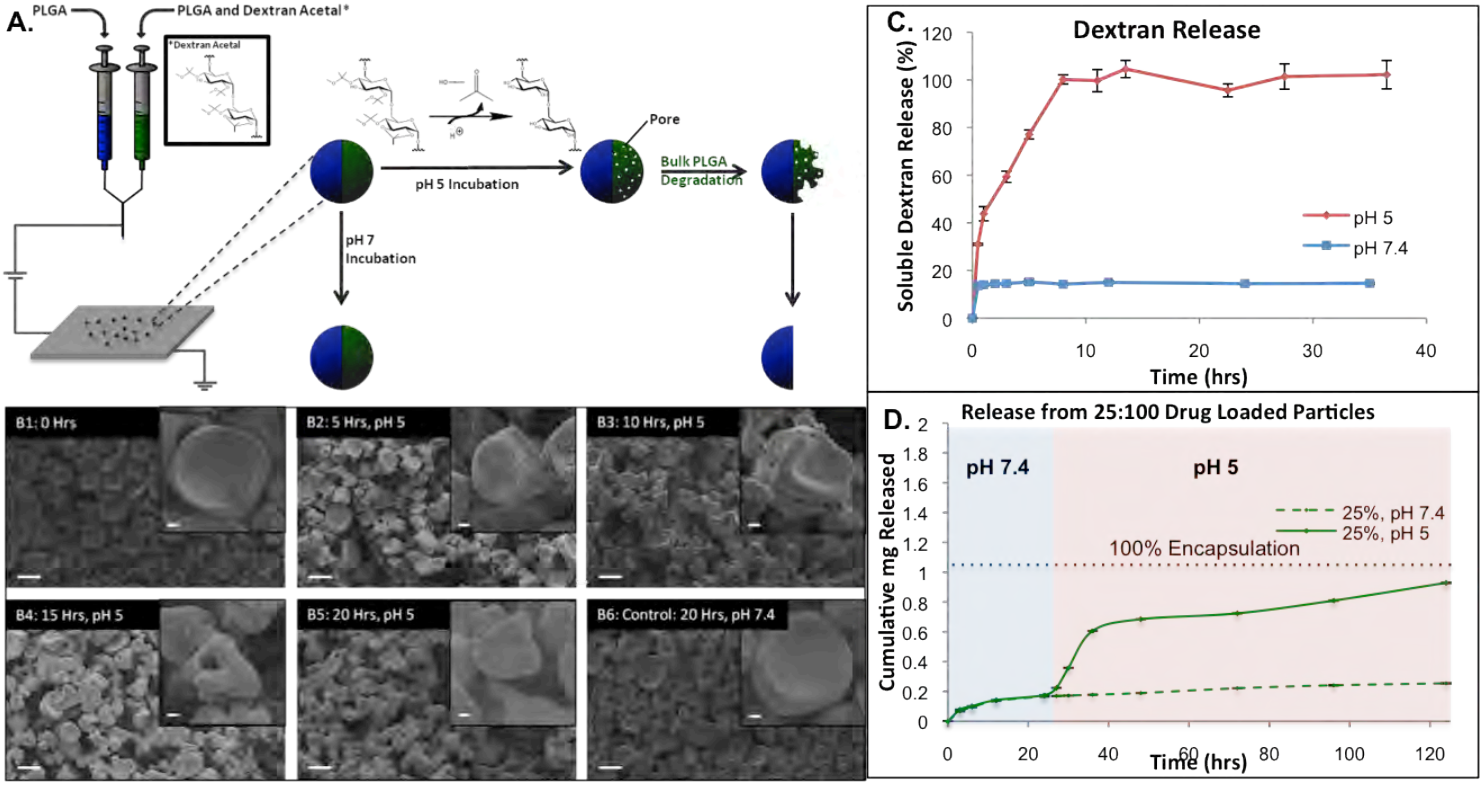
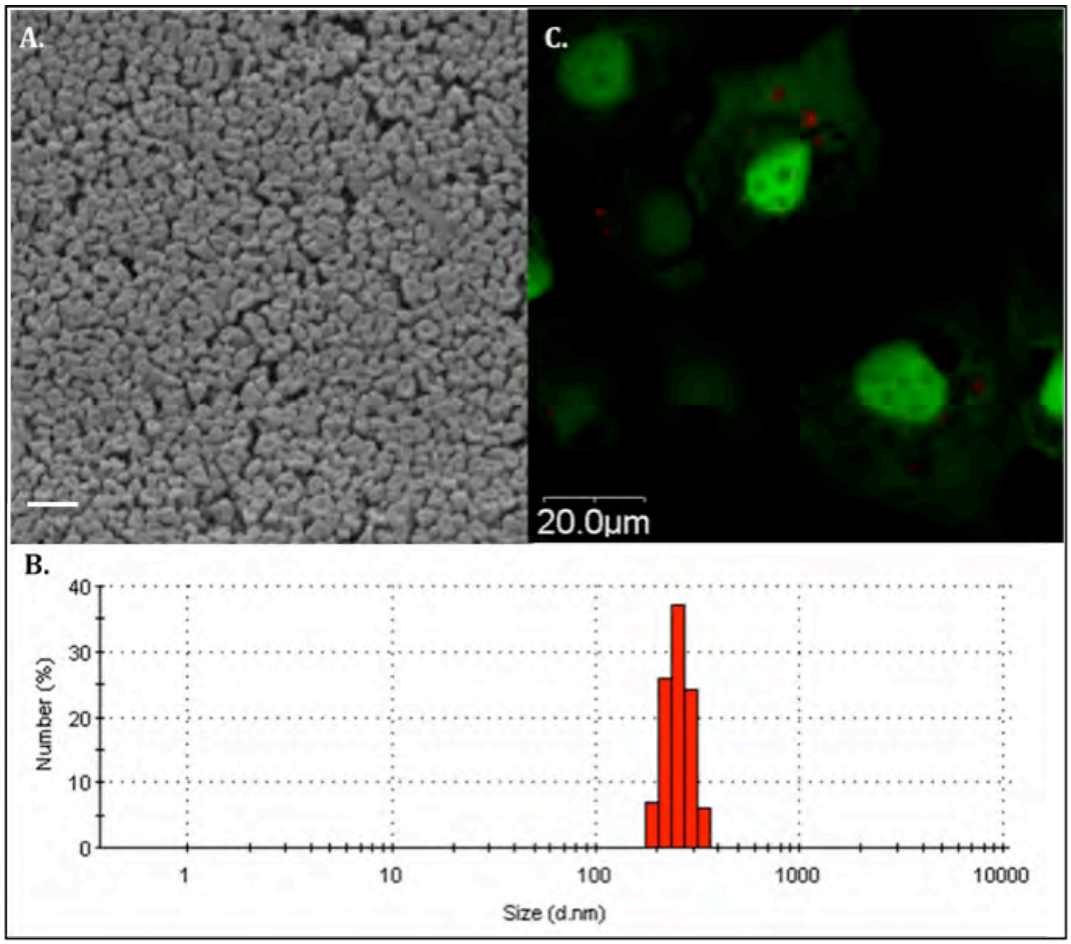


Figure 5: Morphology, degradation, and release from bicompartmental particles containing dextran Acetal (DA). A: Bicompartmental particles containing DA in one compartment are fabricated through the EHD co-jetting procedure. The particles are pH responsive. Upon incubation in pH 5, the DA is deprotected, released, and pores are created on one side of the particles that result in the enhanced degradation of one side. At physiological pH, the DA is not deprotected and the particles do not have any pores. B: SEM images showing a timed study of particle incubation at two different pHs. C: Release of soluble dextran from particles at two different pHs. D: Release of a therapeutic from the particles. Here the therapeutic is encapsulated in the DA containing side and is released once incubated in pH 5 (at the 24 hour mark).

In contrast, when incubated in physiological pH, the particles stay intact (control **Figure 6.B1** is similar to 20 hours incubation at pH 7.4 **Figure 6.B6**). In addition, the release of free dextran was quantified (**Figure 6.C**), which showed that the polymer is released from particles within a 10-hour period in pH 5 (agreeing with the SEM data). Next, particles containing dextran and Irinotecan in one compartment were fabricated and release studies were conducted (**Figure 6.D**). Two sets of particles (four replicas each) were incubated at pH 7.4 for 24 hours and their release kinetics were measured at predetermined intervals (3, 6, 12, and 24 hours). At the 24-hour mark, one set was switched to pH 5 (solid green line) while the other was kept at pH 7.4 (dotted line). While there was minimum release for the set kept at pH 7.4, there was a rapid release of Irinotecan from the set in pH 5 as the dextran was deprotected and released. A slower release of the Irinotecan follows this rapid release, which is due to the fraction of the drug encapsulated in the PLGA component of the compartment.



In order for such on-demand particles to be clinically applicable, they must be nano-sized and monodispersed. Nanoparticles containing DA in one compartment were fabricated through the EHD process by changing the solvents used during jetting. The particles were then separated into the relevant size ranges using serial centrifugation. As shown in **Figure 7.A-B**, the particles are monodispersed as seen in SEM and quantified by Dynamic Light Scattering (DLS). These particles were incubated with breast cancer cells and their cellular uptake was visualized. **Figure 7.C** shows the results of the uptake studies: here the cells autofluoresce green (they have been modified by a GFP gene) and the nanoparticles contain a red dye. Based on preliminary results, it appears that the particles are taken up through endocytosis. It can be projected that at this point due to the low acidic pH, such particles would release their cargo into the cellular environment.

Figure 7: Monodispersed Nanoparticles containing DA in one compartment and their cell uptake. A and B: SEM and DLS of bicompartmental particles with an average size of 200-300 nm. C: Cellular uptake of the Nanoparticles through endocytosis. Here, the nanoparticles contain a red dye and the breast cancer cell line autofluoresce green (GFP).

Key Research Accomplishments.

i. Identification and evaluation of suitable polymer combinations for bicompartmental carriers

ii. Specfic and independent degradation and release of bicompartmental nanoparticles

iii. Synthesis of particles with suitable size and polydispersity

Reportable Outcomes.

MD. Ph.D. student Ashish Misra and Ph.D. student Sahar Rhameni will advance this fall to Ph.D. candidacy in Biomedical Engineering based on their work in this project.

References:

1. Roh, K.H., D.C. Martin, and J. Lahann, *Biphasic Janus particles with nanoscale anisotropy.* Nature Materials, 2005. **4**(10): p. 759-763.
2. Bhaskar, S., et al., *Spatioselective Modification of Bicompartmental Polymer Particles and Fibers via Huisgen 1,3-Dipolar Cycloaddition.* Macromolecular Rapid Communications, 2008. **29**(20): p. 1655-1660.
3. Lahann, J., *Recent progress in nano-biotechnology: compartmentalized micro- and nanoparticles via electrohydrodynamic co-jetting.* Small, 2011. **7**(9): p. 1149-56.
4. Bhaskar, S., et al., *Towards designer microparticles: simultaneous control of anisotropy, shape, and size.* Small, 2010. **6**(3): p. 404-11.
5. Bhaskar, S. and J. Lahann, *Microstructured Materials Based on Multicompartmental Fibers.* Journal of the American Chemical Society, 2009. **131**(19): p. 6650-+.
6. Bhaskar, S., et al., *Multicompartmental Microcylinders.* Angewandte Chemie-International Edition, 2009. **48**(25): p. 4589-4593.
7. Bhaskar, S., et al., *Engineering, Characterization and Directional Self-Assembly of Anisotropically Modified Nanocolloids.* Small, 2011. **7**(6): p. 812-819.
8. Taylor, G., *Disintegration of Water Drops in Electric Field.* Proceedings of the Royal Society of London Series a-Mathematical and Physical Sciences, 1964. **280**(1380): p. 383-+.
9. Roh, K.H., D.C. Martin, and J. Lahann, *Triphasic nanocolloids.* Journal of the American Chemical Society, 2006. **128**(21): p. 6796-6797.
10. Mitragotri, S. and J. Lahann, *Materials for drug delivery: innovative solutions to address complex biological hurdles.* Adv Mater, 2012. **24**(28): p. 3717-23.
11. Bachelder, E.M., et al., *Acetal-derivatized dextran: an acid-responsive biodegradable material for therapeutic applications.* J Am Chem Soc, 2008. **130**(32): p. 10494-5.